Supporting Information:

Anisotropy of pairwise interactions between hexadecanes in water measured by AFM force spectroscopy

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This Supporting Information provides a detailed description of the data manipulation and includes all of the histograms and fits referred to in the main text. Also included are additional details for the synthesis and preparation of the middle-attached hexadecane.

Data Collection and the Extended Freely Jointed Chain Fit:

Upon collection, force curves are saved as waves in Igor Pro 5 (Wavemetrics Inc., Portland, OR). These waves are processed with a custom program written in Matlab (MathWorks, Inc., Natick, MA). The majority of the collected force curves do not exhibit characteristic separation events. Data processing is automated to remove user bias and speed up the data analysis. The program converts the force curves from deflection versus displacement into force versus separation. The deflection noise value is calculated as a standard deviation from the off-surface

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part of the approach line, and the mean noise value based threshold is used to detect rupture events. A threshold of five standard deviations is applied to the retract curve to detect the abrupt transitions in force. After this simple analysis, a filter is applied to remove the rupture transitions that occur at separations too close to the surface (<10 nm). A second set of filters refines parameters prior to the freely jointed chain fit to select force events that have a force pattern typical for separation events coupled to a stretching polymer (when gradual tensioning of the polymeric tether is followed by the abrupt release of the accumulated stress). Our tests indicate that this initial processing does not eliminate force curves that can be considered for further analysis by a trained user. The selected polymer-stretching events are fit with the extended freely jointed chain model [1] to extract the contour length and Kuhn length parameters and determine the loading rate for each separation event. This model is an extension of the commonly used freely jointed chain (FJC) model. The FJC model predicts extension of the polymer chain $x(F)$ with the Kuhn length l_k and contour length L_c as a function of applied force F according to

$$x(F) = L_c \cdot \left[\coth\left(F \cdot l_k \Big/ k_B \cdot T \right) - k_B \cdot T \Big/ F \cdot l_k \right] \qquad \text{(S1)}$$

where k_B is the Boltzman's constant and T is the absolute temperature. This equation is usually written as $x(\beta) = L_c \cdot L(\beta)$ were $L(x)$ is the Langevin function and $\beta = F \cdot l_k/(k_B \cdot T)$.

Besides an entropic elasticity of the polymer chain included in FJC model, the extended model includes elongation of the PEG chain due to monomer elasticity as well as conformational transition between helical and planar conformations of the PEG chain in aqueous solutions.[1] In

this model, the contour length of stretched polymer consists of the lengths of polymer segments at two different conformations, planar and helical:

$$L_c = N_{planar} \cdot L_{planar} + N_{helical} \cdot L_{helical} \tag{S2}$$

Here N_{planar} and $N_{helical}$ are the numbers of segments in planar and helical conformations respectively. L_{planar} and $L_{helical}$ are the corresponding monomer lengths that are fixed to 3.58 Å and 2.8 Å respectively in our calculations.[1] Contour length defined by equation S2 can be related to the common definition of contour length (the maximum distance between ends of the linear polymer chain) by noting that if $N_{helical}$ and N_{planar} have fixed (force-independent) values then the usual definition of contour length can be applied. The ratio of $N_{helical}$ to N_{planar} depends on applied force according to:

$$\frac{N_{helical}}{N_{planar}} = e^{\Delta G(F)/k_B \cdot T}$$
$$\Delta G(F) = \Delta G_0 - F \cdot \left(L_{planar} - L_{helical} \right) \tag{S3}$$

Here $\Delta G(F)$ is the force-dependent free energy difference between the two states and ΔG_0 is this difference at zero applied load, fixed to 7.48 kJ/mol in our calculations.[1] The overall PEG chain with N monomers the extension is

$$x(F) = N \cdot \left(\frac{L_{planar}}{e^{+\Delta G(F)/k_B \cdot T} + 1} + \frac{L_{helical}}{e^{-\Delta G(F)/k_B \cdot T} + 1} \right) \cdot \left[\coth(\beta) - 1/\beta \right] + N \cdot \frac{F}{K_S} \tag{S4}$$

Here the segmental elasticity K_s provides the chain extension at high loads and is held at 150 N/m[1] and the other parameters are described above. This model was used to fit the force curves with two free parameters: the number of monomers N in the chain and the Kuhn length l_k. The Kuhn length was allowed to vary to obtain a close fit to the data near the separation point. The

tether spring constant was obtained as the slope of the fit curve at the rupture point. It was noted that when both FJC and extended FJC models were used to fit the experimental data, FJC model produced systematically higher tether elasticity values. The systematic error in tether elasticity will propagate in the error in the loading rate that is calculated according to

$$l_r = v\,(k_t^{-1} + k_c^{-1})^{-1} \qquad \text{(S5)}$$

where k_c is the spring constant of the cantilever and v is the velocity of the cantilever base. The extended FJC model fits the stretching curves closely, providing more accurate loading rate determination.[2]

Determination of the Most Probable Force and the Most Probable Loading Rate:

Below the experimental rupture force histograms for each set of the alkanes are shown with the cumulative fit (solid grey line), as well as its components (individual bond component, dotted grey line and two-bond component, dotted black line). The bin size for all force histograms is held the same for all histograms and is equal to 15 pN. The black dash-dotted line is the window function fit, as mentioned above, to account for limited force sensitivity. This window function is scaled by the height of the histogram for clarity. Kinetic parameters from these fits are given in the main text.

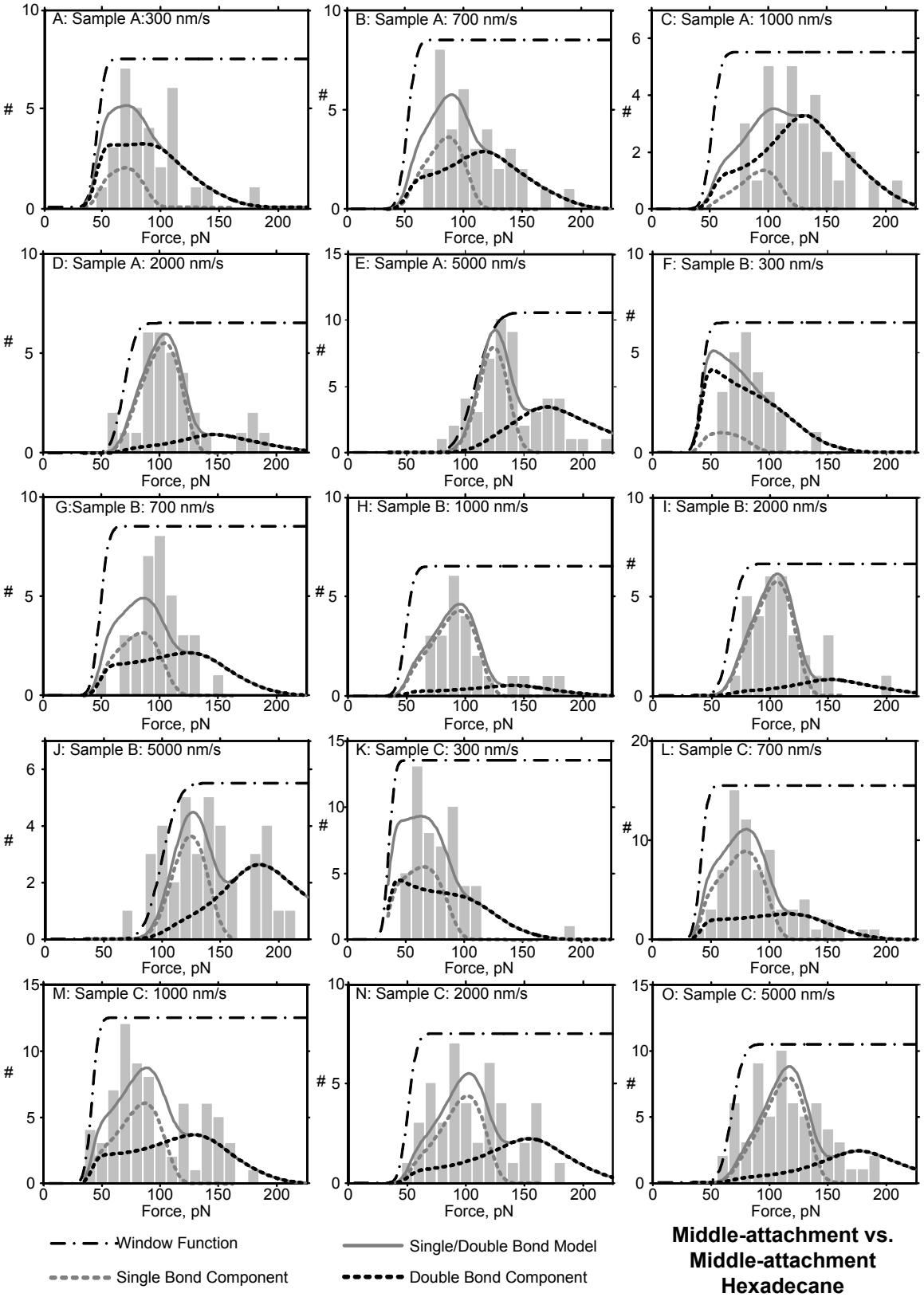


Figure S1. Histograms of rupture forces in the MM configuration fit by the two-bond model.

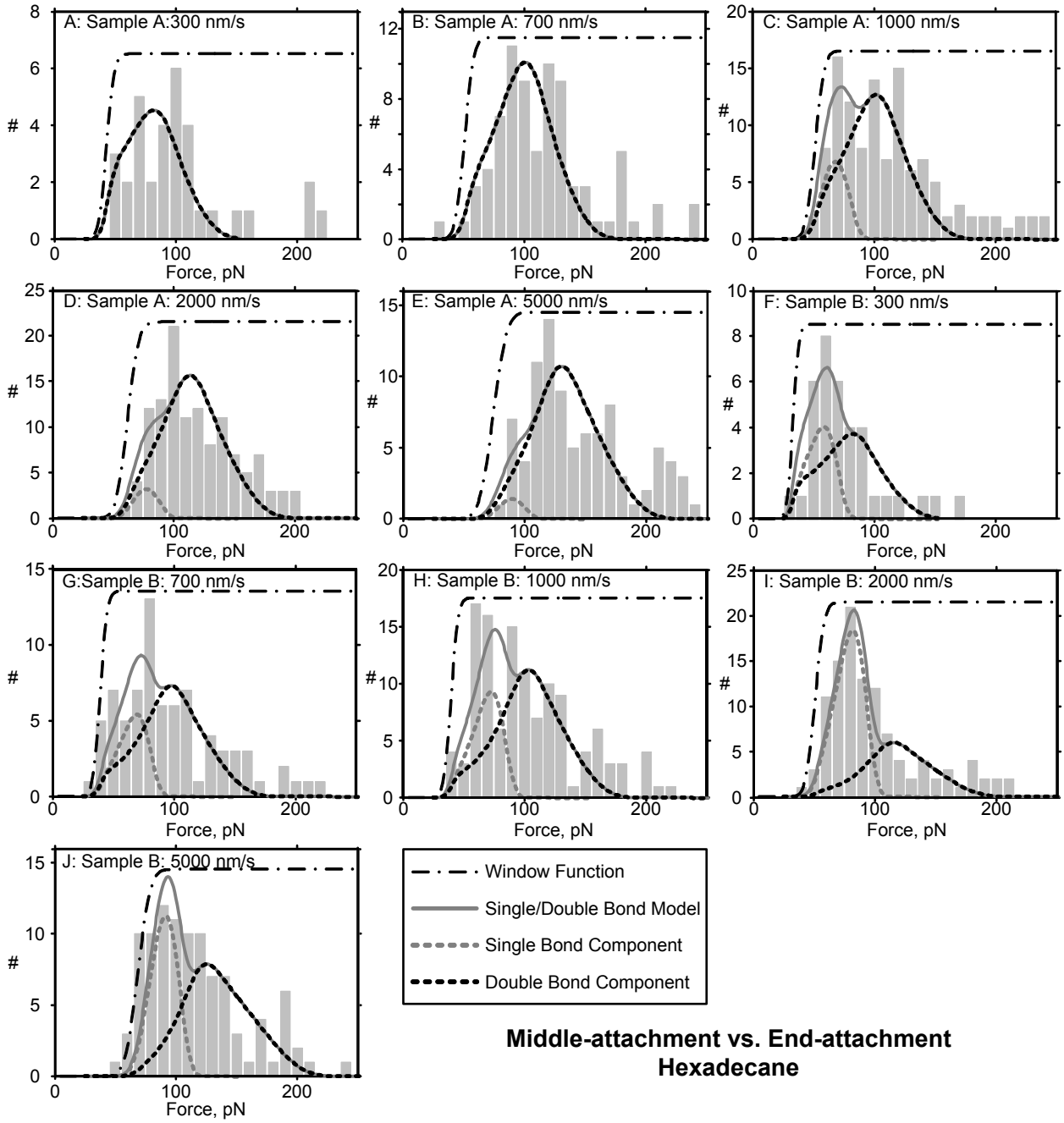


Figure S2. Histograms of rupture forces in the ME configuration fit by the two-bond model.

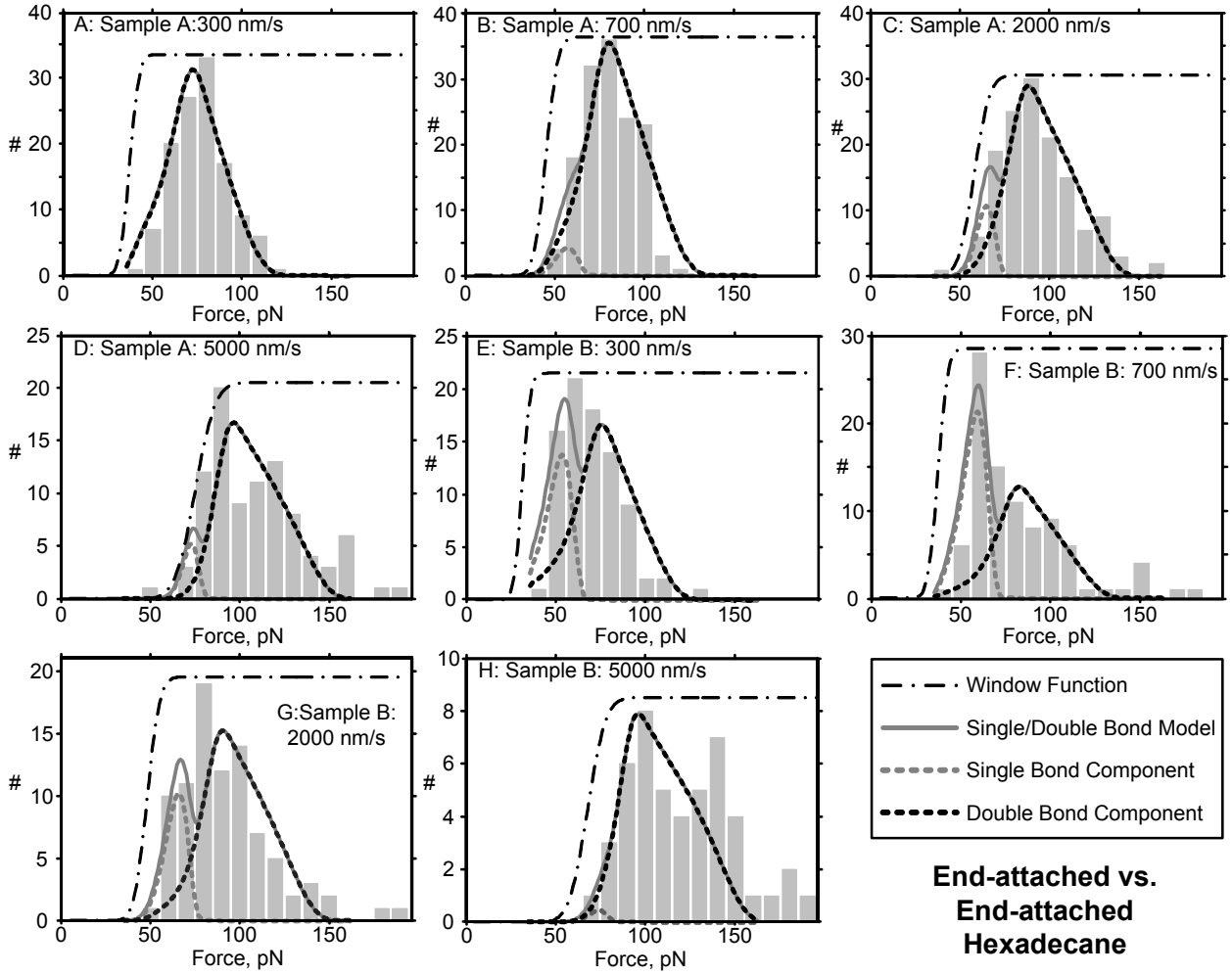
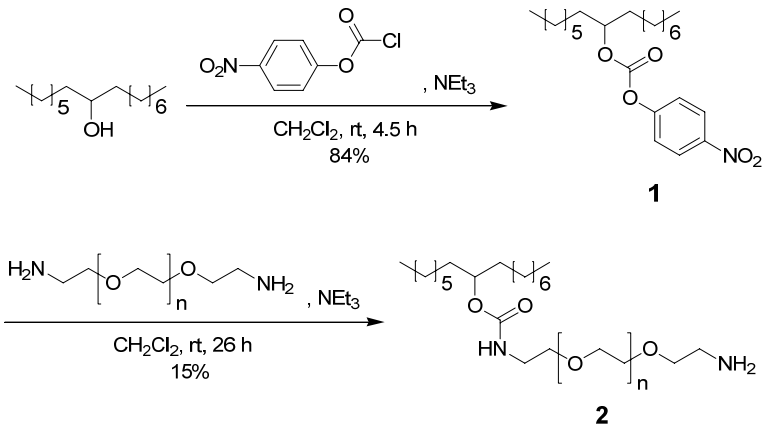


Figure S3. Histograms of rupture forces in the EE configuration fit by the two-bond model.



Scheme 1. Synthesis of M-hexadecane-PEG3350-NH_2 **2**.

Synthesis:

Preparation of Activated M-hexadecane 1.[3] To a solution of 8-hexadecanol 200.0 mg (0.83 mmol) in 5 mL of anhydrous CH_2Cl_2 cooled in ice-water bath, 200.0 mg (1.0 mmol) of 4-nitrophenyl chloroformate and 0.35 mL (2.5mmol) of triethylamine were successively added. The mixture was stirred at room temperature under N_2 for 4.5 h. 15 mL of CH_2Cl_2 was added and the mixture was washed with water (3 × 5 mL) and brine (3 × 5 mL). The organic layer was dried over Na_2SO_4 and filtered. The solution was evaporated to dryness. The resulting residue was purified by column chromatography (hexanes/ethyl acetate 15/1, by volume) to give 284.1 mg (0.70 mmol, 84%) of **1** as an oil. ^{1}H NMR (DMSO-*d*6): δ 8.35 (m, 2H), 7.58 (m, 2H), 4.78 (m, 1H), 1.65 (m, 4H), 1.29 (s, 22H) 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 155.9, 152.5, 145.4, 125.4, 121.9, 81.5, 34.0, 32.0, 31.9, 29.8, 29.6, 29.4, 29.3, 25.3, 22.8, 14.2. MS: *m/z* 408 (MH^+), HRMS calcd (found) for $C_{23}H_{38}N0_5$ (MH+): 408.27497 (408.2748).

Preparation of M-hexadecane-PEG3350-NH_2 2. To 340.0 mg (0.1 mmol) of poly(ethylene glycol) (PEG) diamine in 10 mL of anhydrous CH_2Cl_2, a solution of 40.7 mg of **1** and 15 μL of

triethylamine in 5 mL of anhydrous CH_2Cl_2 was added dropwise at 0 ^oC over 6 h period. After addition of **1** the mixture was stirred at room temperature under N_2 for 20 h and then diluted with 10 mL of CH_2Cl_2. The mixture was washed with water (2 × 5 mL) and brine (1 × 5 mL). The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed *in vacuo*. The resulting residue was subject to column chromatography on silica gel using methanol/CH_2Cl_2 (1/4, by volume) as the eluent to give the crude product, which is further purified by precipitation in cold diethyl ether to afford 53.9 mg (0.015 mmol, 15%) of **2** as a solid. The presence of primary amine group was confirmed by a positive ninhydrin test. ^1H NMR ($CDCl_3$): δ 5.24 (broad, NH's), 5.03 (broad, NH's), 4,65 (m, 1H), 4.16 (m, 4H), 3.80-3.24 (m, 304H), 1.44 (m, 4H), 1.21 (s, 22H), 0.83 (m, 6H).

References

(1) Oesterhelt, F.; Rief, M.; Gaub, H. E. *New J. Phys.* **1999**, *1*, 6.1.

(2) Ray, C.; Akhremitchev, B. B. *J. Am. Chem. Soc.* **2005**, *127*, 14739.

(3) Cai, T. B.; Lu, D.; Tang, X.; Zhang, Y.; Landerholm, M.; Wang, P. G. *J. Org. Chem.* **2005**, *70*, 3518.